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Mechanisms of Hormone Actions on Gill Transport

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I. INTRODUCTION

The gills of fishes represent an extensive surface area (up to 90% of the total body surface) that is in intimate contact with the ambient water. The distance between water and blood is very small. One or two epidermal cell layers, a few micrometers in thickness, separate both fluids.

The gills serve three principal functions: respiration, ion regulation, and excretion of metabolic waste products. The large surface and small thickness favor respiratory gas exchanges and the outward diffusion of waste products, but put substantial osmoregulatory stress on the fish because the gill epithelium is to some extent permeable to water and ions. In the maintenance of hydromineral balance of the body fluids the gills play a pivotal role.

In freshwater teleosts the excretion of large volumes of urine compensates for the water invading the fish by osmosis. Even though urine is highly hypotonic to the blood, it results in ion losses. Urinary and branchial diffusional ion losses are compensated for mainly by the uptake of ions from water. The mechanisms of ion uptake are concentrated in the gills.

Seawater teleosts face dehydration and excessive accumulation of ions. Water losses are compensated for by drinking of water and by limiting urine

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production. The excess of ions, caused by inward branchial ion diffusion and by the entry of ions associated with drinking, is excreted via the gills.

Branchial ion regulation also includes the removal of metabolic end products that are largely independent of the external medium, in particular H^+, NH_4^+, and HCO_3^-, which are partly exchanged for Na^+ and Cl^-. This implies that ion regulation at the level of the gills is closely related to acid–base regulation and respiration. Gills are also a site of excretion of nonionic products. Moreover, in fresh water the major part of the Ca^{2+} required for growth and reproduction enters the fish via the gills.

The manifold functions of the gills are controlled by complex endocrine regulatory mechanisms. The endocrine system of teleosts includes almost all glands and hormones found in terrestrial vertebrates but in addition some others such as the Stannius corpuscles and the caudal neuroendocrine gland, the urophysis. Before studying the mechanisms of the different hormonal actions on the gill, we present a general outline of the structure of the gill and of the branchial exchanges with the external medium in relation to two principal biotopes: freshwater and seawater.

II. STRUCTURE AND VASCULARIZATION OF THE GILLS

The branchial apparatus of teleost fishes consists of four pairs of gill arches. From each gill arch two rows of filaments extend, and each of these carries densely packed lamellae (commonly 30–40 per millimeter). The tips of filaments from adjoining arches meet and make a basket-like system so that water flows between the lamellae in one direction and the blood in the lamellae in the opposite direction (see reviews by Hughes, 1984; Laurent, 1984).

A. Filaments

The filaments are covered by a multilayered epithelium of pavement cells, which is also called primary or interlamellar epithelium. It is in contact mainly with the central venous system of the filaments. Between the pavement cells, so-called chloride cells (C.C.) or ionocytes are present, which, since their first description by Keys and Wilmer (1932), have been studied intensively (Laurent, 1984). They are present in freshwater as well as seawater fish, but changes in these cells have been observed when euryhaline fishes migrate between fresh water and seawater. In freshwater fishes, the C.C. are in general isolated cells, only occasionally accompanied by accessory cells (slender, probably immature chloride cells), and surrounded by pavement or respiratory cells, to which they are apically linked by multistranded tight junctions. In some species the C.C. form an apical crypt, but this is often absent in freshwater fishes. The cells typically contain many

mitochondria and a well developed system of membrane tubules, the lumens of which are continuous with the extracellular space. In seawater fishes, usually two or more C.C. are located together and share the same apical crypt. More frequently than in freshwater fishes, the cells are accompanied by accessory cells (Fig. 1). In some seawater species it has been demonstrated that the C.C. are interconnected by single-stranded junctions that constitute relatively leaky paracellular pathways, while they are linked to respiratory cells by multistranded tight junctions (Sardet *et al.*, 1979).

In some freshwater and seawater fish C.C. are also present outside the gill arches, e.g., in the skin covering the buccal side of the opercula or other parts of the body. These skin areas present excellent objects for experimental studies on C.C. functions (e.g., Marshall, 1977; Foskett *et al.*, 1981; Zadunaisky, 1984).

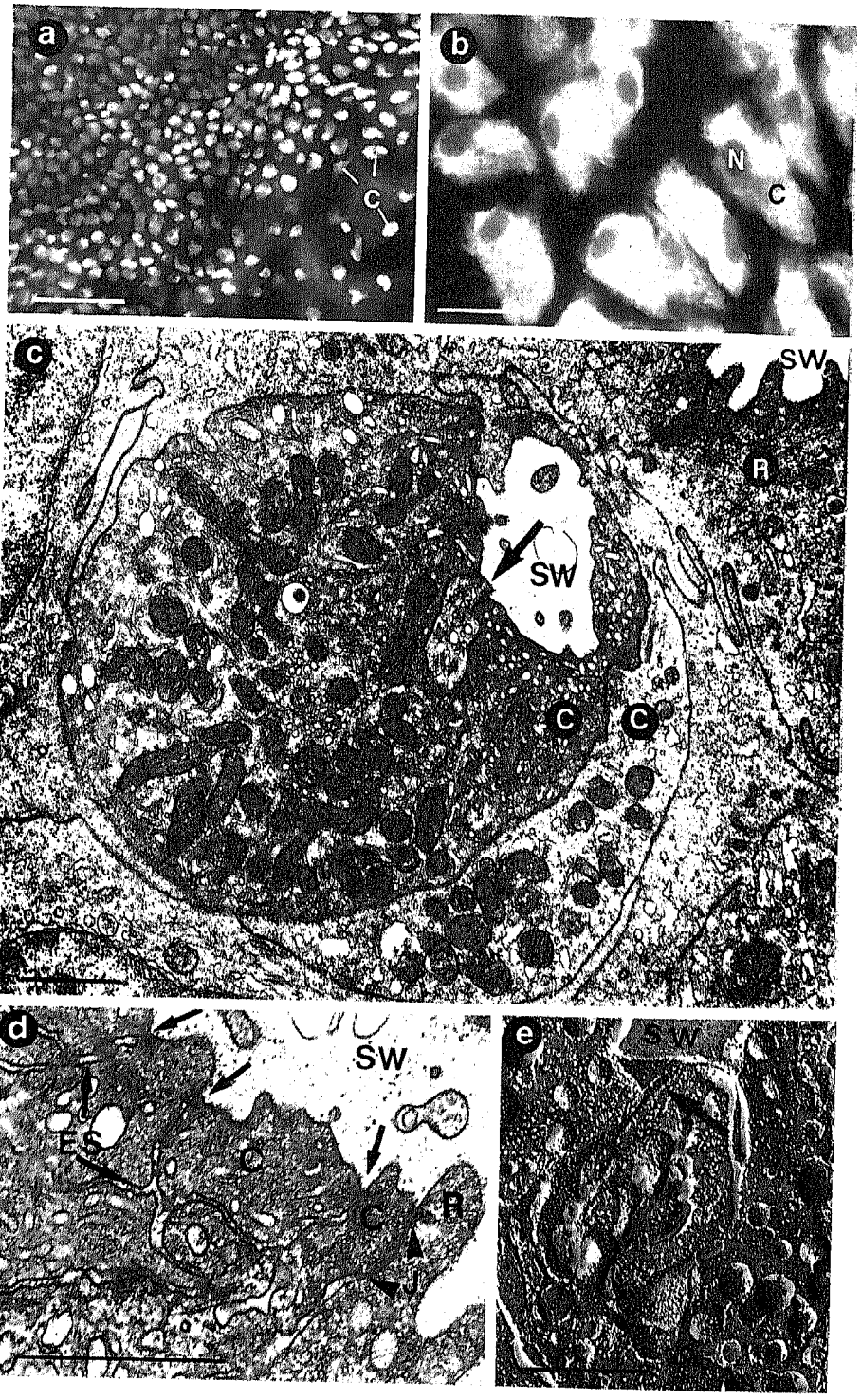
B. Lamellae

The lamellae make up most of the surface area of the gill. They are covered with one or two layers of respiratory cells. These epidermal cells are linked to each other by tight junctions. They cover the central blood spaces of the lamellae. So-called pillar cells that function as specialized endothelial cells interconnect the opposing sheet of respiratory cells of the lamellae and may control the blood flow through marginal and basal vessels (Fig. 2).

In their original description of the C.C., Keys and Willmer (1932) reported the presence of C.C. on both the filaments and lamellae. More recently, C.C. have been reported to be absent from the lamellae of several fish species, including trout and eel (Sardet *et al.*, 1979). However, recently it has been shown that C.C. are abundant on the lamellae of trout from fresh water with very low or very high Ca^{2+} concentrations (Laurent *et al.*, 1985; Perry and Wood, 1985; Avella *et al.*, 1987). We have observed C.C. on lamellae of trout from fresh water with rather high Ca^{2+} concentrations (0.8–1.6 m*M*; Fig. 2). Laurent and Dunel (1980) reported the presence of C.C. on the lamellae of fish affected by wounds or fungal diseases. Thus, the distribution of C.C. may be dependent on the presence or absence of stress factors or with the composition of the water. In several other species, including American and European eels and tilapia (*Oreochromis mossambicus*), the C.C. occur on filaments and on lamellae, although most are located on the former.

C. Vascularization

With respect to the blood supply of the gills, minor variations between species have been reported (Laurent, 1984), but differences between seawater- and freshwater-adapted specimens of a single species are not known to us. The circulation of the gills is in series with the systemic circulation and, since the gills are the first organ after the heart, the branchial arterial pressure is maximal (30–40 mm Hg). However, by the combined activity of pillar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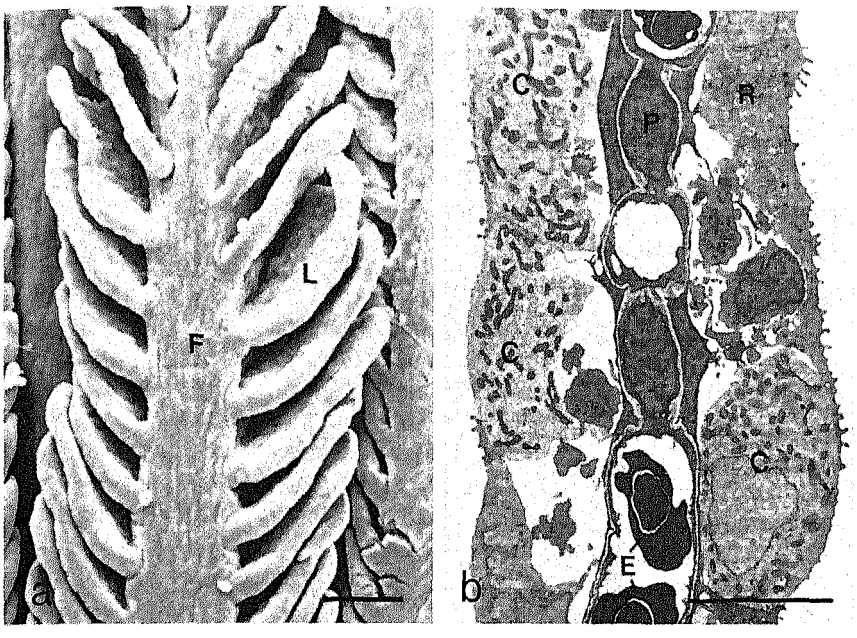

Fig. 2. Fish from fresh water with high Ca^{2+} concentration (0.8–1.6 m*M*). (a) Scanning electron micrograph of a gill filament of rainbow trout, showing filament (F) and lamella (L). (b) Cross section of a branchial lamella of rainbow trout, showing respiratory cells (R), chloride cells (C), pillar cells (P), and erythrocytes (E) (transmission electron micrograph; ×6560).

Fig. 1. Seawater fish. Opercular membrane of the killifish, *Fundulus heteroclitus*. (a) Surface of opercular epithelium after incubation with D.A.S.P.M.I. (dimethyaminostyrylmethylpyridiniumiodine). Chloride cells (C.C.) fluoresce intensely (epifluorescence microscopy; ×120). (b) Detail of D.A.S.P.M.I.-treated opercular membrane. The fluorescence is localized in the cytoplasm, and is due to the large mitochondrial space. Nuclei (N) are negative (epifluorescence microscopy; ×980). (c) Ultrathin section of opercular membrane, showing two C.C. (C) with their apical cavity (which is actually in open connection with the seawater (SW) lying below respiratory cells (R). Arrow indicates cytoplasmic process of one C.C. penetrating into the neighbouring C.C. (transmission electron micrograph; ×13,000). (d) Detail of apical region of epithelium facing seawater (SW). Interdigitations of neighboring C.C. are shown, separated by narrow extracellular spaces (ES). Shallow tight junctions, between C.C. cells, are shown by arrows. Note the deep tight junction (J) between chloride cell and respiratory cell (transmission electron microscopy; ×28,700). (e) Cytoplasmic process of one C.C. penetrated into a neighboring C.C. Arrow indicates a one-stranded tight junction between the C.C. (freeze-fracture electron micrograph; ×35,300). The authors are grateful to Dr. C. Sardet for providing the picture.

cells and sphincters at the entrance and at the exit of the central venous system (Fig. 3), the secondary lamellae and the interlamellar region can be perfused differently. For example, it has been shown that in the trout the blood reaching the central venous vessels is oxygenated (efferent) while in the eel the central venous vessels will receive mixed (afferent and efferent) blood. This is of great importance when the hormonal control of ion exchange of the gills is considered.

III. ION TRANSPORT ACROSS THE GILLS

For understanding of hormonal actions on the gills it is important to consider the kind of ion movements that occur, the specific cell types involved, the cellular membranes in which the ion translocating mechanisms are located, and the importance of the paracellular pathway for ion transport. Ion transport across the gills of teleost fish has been reviewed extensively in the last 15 years (e.g., Maetz, 1974; Evans, 1984; Zadunaisky, 1984; Payan *et al.*, 1984). Here only a summary is presented for freshwater and seawater fishes.

A point to keep in mind is the tremendous difference that often occurs between the magnitude of ion movements through the gills of seawater fishes (from 100 to 12,000 μeq/hr per 100 g) and freshwater fishes (from 2 to 140 μeq/hr per 100 g). The net ion flux often is a small percentage of the unidirectional fluxes, especially in seawater.

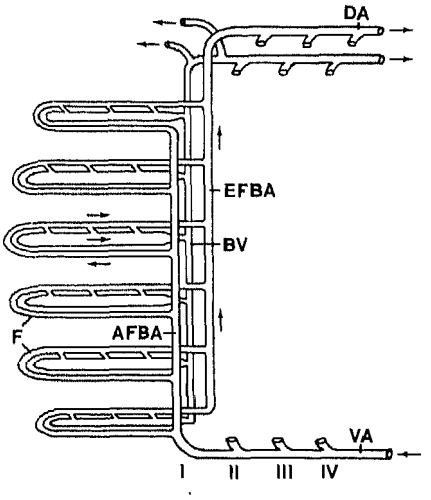

Fig. 3. Diagram of a single hemibranch. I, II, III, IV are gill arches; F, filaments (between 50 and several hundred per gill arch); DA, dorsal artery; VA, ventral artery; AFBA, afferent branchial artery; EFBA, efferent branchial artery; BV, branchial vein. The arrows indicate the blood flow [see Hughes (1984) and Laurent (1984) for details].

A. Freshwater Fishes

Early studies on intact fishes and more recent experiments in which the branchial region was separated from the rest of the body (isolated gill arches; isolated-head techniques) have clearly demonstrated that there is an active ion uptake across the gill epithelium.

The transepithelial potential (TEP) of the gills (up to 30 mV, inside negative) results from differential ionic permeability and from electrogenic ion uptake (Maetz, 1974).

The uptake of Na^+ and Cl^- from the water must have an active component as it occurs against chemical gradients.

There is evidence that Na^+ uptake partly takes place via Na^+/H^+ and Na^+/NH^+ exchange. In isolated trout-head preparations, pH changes in the perfusion fluid induce changes in Na^+ entry. Both Na^+ uptake and H^+ efflux are inhibited by amiloride, an Na^+-channel blocker. There is an inverse correlation between Na^+ uptake and the acidification of the water, and metabolic acidosis leads to increased Na^+ uptake. The data point to the presence of electroneutral carriers at the apical cell membranes.

Crossing of Na^+ through the basolateral cell membrane of the branchial epithelium probably occurs via Na^+,K^+-ATPase, as Na^+ entry into the blood is sensitive to ouabain. In isolated trout-head experiments it has been shown that Na^+ entry is correlated with the presence of K^+ in the perfusion fluid (Richards and Fromm, 1970; Shuttleworth and Freeman, 1974). Recently, some evidence for the presence of Na^+,H^+-ATPase activity has been reported in the gills of tilapia (Balm, 1986). The enzyme activity copurifies with Na^+,K^+-ATPase and is stimulated by NH_4^+.

Na^+ and Cl^- can be extracted independently from the water. Kerstetter and Kirschner (1972) have proposed a Cl^-/HCO_3^- exchange. A microsomal anion-ATPase was shown to be present in the gill with kinetic parameters compatible with the branchial Cl^- uptake (De Renzis and Bornancin, 1977). Carbonic anhydrase participates, at least in some species, in the production of H^+ and HCO_3^-, as acetazolamide treatment is accompanied by a fall in Na^+ and Cl^- uptake (Payan *et al.*, 1975).

The cell type that contributes to Na^+ and Cl^- uptake in fresh water is still under discussion. Both respiratory cells and C.C. may participate in ion entry, depending on internal needs to extrude H^+ and NH_4^+ and depending on the water composition (Payan *et al.*, 1984; Gardaire *et al.*, 1985).

Ca^{2+} exchanges take place through the gill, and the C.C. can be considered as the principal site of Ca^{2+} entry. Recently a high-affinity Ca^{2+}-ATPase has been identified in the gill and was shown to be the driving force for Ca^{2+} transport across the plasma membranes (Flik *et al.*, 1984a, 1985). This enzyme copurifies with Na^+,K^+-ATPase (Flik *et al.*, 1984b) and is therefore most likely located in the C.C.

B. Seawater Fishes

In general, the following have been shown for seawater fishes.

The gill is the principal site for Cl^- extrusion. Foskett and Scheffey (1982), using the vibrating probe technique, were able to localize the current and conductance pathways in opercular epithelium and demonstrated that the C.C. are the sites of Cl^- extrusion. Cl^- extrusion is electrogenic and is sensitive to isocyanate, a competitive inhibitor of Cl^- transport sites. While Cl^- transport occurs transcellularly, Na^+ probably follows the paracellular route between the C.C. (Karnaky, 1986). Cl^- extrusion is dependent on Na^+,K^+-ATPase as its driving force. It is inhibited by ouabain (see Zadunaisky, 1984). Bicarbonate stimulates the short-circuit current, TEP, and Cl^- fluxes in opercular membranes of *Fundulus heteroclitus*, when introduced to the inner side (Degnan *et al.*, 1977). In isolated eel C.C. it could be demonstrated that Cl^-- and HCO_3^--stimulated ATPase activity was three times higher than in isolated respiratory cells (Naon and Mayer-Gostan, 1983). More studies are necessary to establish the identity of this enzyme and its function in Cl^- transport.

The mechanisms behind the transepithelial Na^+ movements are still controversial. Na^+ efflux probably follows the paracellular route. In many euryhaline seawater species Na^+ is in electrochemical equilibrium across the gills. However, in some stenohaline seawater fishes the TEP is maintained far below the Na^+ equilibrium potential and Na^+ probably is actively extruded (Evans, 1980; Potts, 1984).

Many, although not all, euryhaline fishes show higher branchial Na^+,K^+-ATPase activity when in seawater. Histochemical and autoradiographical studies have shown that the Na^+,K^+-ATPase is located in the membranes of the basolateral tubular invaginations of mature C.C.

Na^+ extruded from the cell by Na^+,K^+-ATPase moves out extracellularly and passively, at least when the TEP is more inside positive than the equilibrium potential for Na^+ (Zadunaisky, 1984). C.C. were also shown to contain carbonic anhydrase in their cytoplasm (Lacy, 1983), but the role of carbonic anhydrase in ion exchanges has not yet been clarified.

Na^+/NH_4^+, Na^+/H^+, and Cl^-/HCO_3^- exchanges, present in freshwater fishes, have also been observed in seawater fishes (Evans, 1984). They are important for acid–base and pH regulation, but will aggravate the accumulation of Na^+ and Cl^- in seawater fishes. However, their contribution to total branchial ion entry is very small compared to the diffusional components.

An important part of total ion movements in the gills of seawater fishes may be represented by Na^+/Na^+ and Cl^-/Cl^- exchange diffusion. The carriers concerned have been proposed to be located in the respiratory cells (see Payan *et al.*, 1984). However, the existence of these exchanges, which would have no importance for the net hydromineral balance of fishes, has

been questioned for C.C. (Degnan and Zadunaisky, 1980; Zadunaisky, 1984).

IV. HORMONAL EFFECTS ON GILLS

A. Catecholamines

Under resting conditions the plasma levels of epinephrine and norepinephrine in teleosts amount to about 1 n*M* (Le Bras 1984; Woodward, 1982; Ling and Wells, 1985). Dopamine appears to be present in only very small amounts in teleosts (Ristori *et al.*, 1979; Le Bras, 1984). The relative amounts of epinephrine and norepinephrine may vary between species, and plasma catecholamine (CA) concentrations may be influenced by the season (Le Bras, 1984). Recent observations by Nekvasil and Olson (1985) indicate that the gills show specific binding of circulating CA (especially norepinephrine) and might be important in regulating plasma levels of norepinephrine. CA release in fish can be evoked by many stimuli, including handling stress (Pickering, 1981). Exposure of trout to low water Ca^{2+} levels results in an immediate although transient increase of plasma epinephrine levels, whereas the norepinephrine levels are unaffected (Perry *et al.*, 1987). Controversy still exists on the relation between exercise and plasma CA levels (Butler and Metcalfe, 1985). As will be shown in this section, high plasma levels of CA exert effects on blood flow through the gills, on active ion transport mechanisms, and on branchial permeabilities to water, ions, and organic substances.

1. Control of Branchial Vasculature

Studies on intact fishes, perfused isolated heads, or perfused gill arches have shown a bimodal response to CA: a transient vasoconstriction is first observed (Wood, 1975), followed by a prolonged vasodilation. This response occurs in freshwater fishes (Payan and Girard, 1977) as well as in seawater fishes (Claiborne and Evans, 1980). Vasoconstriction alone appears after β-receptor blockade while α-blockade produces only vasodilation. There is a decrease of the branchial resistance after epinephrine (Bolis and Rankin, 1980). The threshold of responsiveness of vessels to epinephrine was shown to be very low (10^{-11}–10^{-12} *M*) (Ristori, 1984). CA probably act at multiple sites, including the sphincter located on the efferent artery. This sphincter, which also receives nerve terminals, can be contracted by cholinergic (muscarinic) agonists (Wood, 1975) and dilated by β-adrenergic agonists (Dunel and Laurent, 1977). Another structure of a sphincter type is present at the entry and exit of the lamellae and might react to circulating CA, as no nerves

have been observed. α-Receptors situated on the arterio–venous anastomosis could be responsible for the decrease in blood flow in the central venus sinus, favoring an increase of blood flow toward the lamellae (Girard and Payan, 1976; Rankin and Babiker, 1981; Claiborne and Evans, 1980). The main result of CA appears to be greater blood flow through the lamellae and enlargement of the functional respiratory surface, possibly in part by lamellar recruitment (Fig. 4). This may effect a 4- to 13-fold increase in oxygen uptake and CO_2 excretion (see review by Nilsson, 1984).

2. *CA Effects on Ion Fluxes in Freshwater Fish*

Payan *et al.* (1975), using the perfused trout head, observed a β-mediated stimulation of Na^+ influx, whereas Na^+ efflux remained unaffected. Contrastingly, in free-swimming trout an increase of Na^+ efflux was found without an effect on Na^+ influx (Wood and Randall, 1973). The same situation appears to exist for Cl^- uptake. Perry *et al.* (1984) observed in the isolated head of the trout an α-mediated stimulation of Cl^- influx by epinephrine, while more recently, S. F. Perry (personal communication) observed that infusion of epinephrine (5×10^{-8} *M*) inhibits both Na^+ and Cl^- uptake *in vivo*. In vitro results from Payan *et al.* (1981) showed that epinephrine stimulates Ca^{2+} uptake via β-adrenergic receptors, while Perry *et al.* (1987), using continuous infusion *in vivo*, observed no adrenergic effects on branchial Ca^{2+} uptake. Basic adenylate cyclase activity in trout gills was shown to be significantly stimulated by isoproterenol (10^{-6} *M*) with an optimal temperature between 20 and 30°C (Guibbolini, *et al.*, 1985).

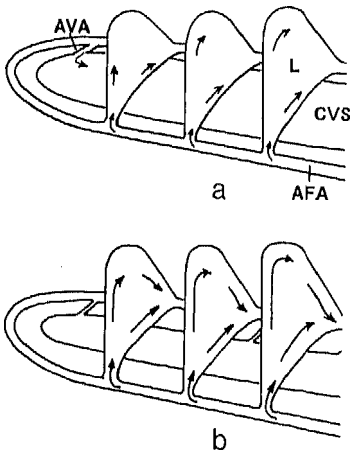

Fig. 4. Diagram of blood flow through a branchial filament illustrating how adrenalin (b) may alter the distribution of blood flow (a) in the gill: AFA, afferent filamental artery; EFA, efferent filamental artery; CVS, central venous sinus; AVA, arteriovenous anastomosis; L, lamella.

3. CA Effects on Ion Fluxes in Seawater Fishes

Keys and Bateman (1932) first showed that in the heart–gill preparation of the eel, epinephrine increased the Cl^- concentration in the perfusion fluid. Pickford *et al.* (1971) and Pic (1972), in killifish and in the mullet, respectively, observed an increase of plasma osmolarity resulting from an increased water loss induced by β-adrenergic receptor stimulation (Pic *et al.*, 1974) associated with a decrease in Na^+ and Cl^- extrusion via α-adrenergic receptor stimulation (Pic *et al.*, 1975). Entry of Na^+ and Cl^- through the filaments is not modified by epinephrine. Entry of Na^+ through the gill is stimulated (via β-receptors), and this is interpreted as a stimulation of the Na^+/NH^+ and Na^+/H^+ exchanges in seawater-adapted fish (Payan and Girard, 1978). Entry of Cl^- is inhibited via α-receptors, and this is interpreted as a decrease of the exchange diffusion component. Research in this field and on the influence of CA on seawater osmoregulation has been advanced significantly by the development of *in vitro* techniques using isolated skin preparations. All results from such preparations (Mayer-Gostan and Maetz, 1980; Marshall and Bern, 1980; Foskett *et al.*, 1982a) show that epinephrine reduces the short-circuit current and the Cl^- extrusion via α-adrenergic stimulation. Responses are dose-dependent, and epinephrine is effective at concentrations as low as 1 n*M* (Foskett *et al.*, 1983), which suggests physiological regulation of the activity of the C.C. by CA. The significance of a relatively small β-mediated stimulation of active ion fluxes is uncertain. Epinephrine possibly affects the active Cl^- efflux by lowering the cAMP concentration, as observed after α-receptor stimulation in the seawater mullet (Djabali and Pic, 1982). This decrease in cAMP is generally accepted (Foskett *et al.*, 1983; Davis and Shuttleworth, 1985). However, May and Degnan (1984) found an increase of cAMP induced by β-stimulation associated with an increase in short-circuit current. They also observed that an inhibition of the short-circuit current can occur in the presence of a high cAMP level. Epinephrine stimulated the turnover of phosphatidic acid and phosphatidyl inositol in enriched C.C. populations of seawater (but not freshwater) eels.

4. CA Effects on Permeability to Nonelectrolytes

Epinephrine increases the branchial permeability to small molecules such as water and urea, but not to mannitol and dextran. The observed effects are more pronounced in fresh water than in seawater (Isaia, 1979). These permeability increases result from β-adrenergic stimulation. In line with this, Brown and Zadunaisky (1982) showed that in isosmotic conditions, isoproterenol increases the water outflux across the opercular membrane of *Fundulus heteroclitus*. Haywood *et al.* (1977), working with the perfused trout head, concluded that the hormonal effect is not achieved via increased functional surface area of the gill but via increased membrane permeability. The

hormonal effect is considered as an action on the fluidity of the lipid phase of the respiratory cell membrane that facilitates the transfer of molecules, since the increase in permeability is obtained without variations of the apparent activation energies (Isaia, 1984). While epinephrine increases the diffusional permeability to water, it abolishes the rectification phenomena (Isaia, 1979).

B. Prolactin

Prolactin has many actions in vertebrates (Bern, 1975; Clarke and Bern, 1980). In the aquatic vertebrates, however, its most prominent effects relate to hydromineral control, with gut, kidney, and, in particular, the gills as target organs. Most important is the reduction by prolactin of the permeability to water and ions of the branchial epithelium of freshwater fishes (Bern, 1975; Hirano, 1977). Prolactin can further modify the activity of some ion-transporting ATPases (Pickford *et al.*, 1970), and it can stimulate growth and differentiation of the branchial epithelial cells. In all freshwater teleosts studied so far, hypophysectomy results in a decline of plasma electrolytes that may be fatal in many species. The drop in plasma electrolytes following hypophysectomy is in general caused partially by renal losses and partially by increased permeability of the gills to water and ions. Both effects are countered by injection of prolactin, which leads to partial or complete restoration of plasma electrolyte concentrations (Dharmamba and Maetz, 1972; Pang *et al.*, 1973, 1978; Ogawa *et al.*, 1973). As presented in Section I, Ca^{2+} uptake takes place in the gill of freshwater fish. Prolactin was shown to induce hypercalcemia in several fish species (Pang *et al.*, 1978; Wendelaar Bonga and Flik, 1982; Flik *et al.*, 1984b), and it has been reported that it stimulates Ca^{2+} influx across perfused eel gills (Ma and Copp, 1981). Recently, Flik *et al.* (1984b, 1986) have shown that prolactin treatment of eels and tilapia increases the high-affinity Ca^{2+}-ATPase activity in the gills and stimulates the Ca^{2+} influx, while Na^+,K^+-ATPase and Ca-dependent phosphatase activities remained unchanged. The density of branchial C.C., which are the presumptive location of these enzymes, increased slightly and significantly (G. Flik and S. E. Wendelaar Bonga, personal communication). Determinations on gills from fishes pretreated with prolactin invariably indicate a reduction of the osmotic permeability to water when studied *in vitro* (Ogawa, 1975, 1977; Wendelaar Bonga and Van der Meij, 1981). Ogasawara and Hirano (1984) reported that a high external Ca^{2+} concentration (> 1 m*M*) may mask the prolactin effect. Measurements of water turnover rates *in vivo* showed that prolactin increases the low turnover rate that is characteristic for hypophysectomized fishes (Potts and Fleming, 1970), and it has been concluded that prolactin increases the branchial osmotic diffusional permeability to water. However, the disturbed water balance of hypophysectomized fishes is a complicated syndrome, and it is questionable whether conclusions with respect to branchial permeability are allowed. The results of the *in vitro* techniques for the estimation of water permeability should also

be interpreted with some reservation (see review by Rankin and Bolis, 1984). In seawater fishes, prolactin cell activity is low, and this is reflected by low plasma prolactin levels (Nicoll *et al.*, 1981; Hirano *et al.*, 1985; Prunet *et al.*, 1985). Activation of prolactin secretion, observed during seawater to fresh water transfer of euryhaline fishes, may facilitate osmoregulatory adaptation to the new environment. In seawater-adapted *Fundulus kansae* (Potts and Fleming, 1971) and tilapia (Clarke, 1973) prolactin injections increase plasma electrolyte concentrations. Prolactin dramatically reduces the high Na^+ turnover rate, which is typical of seawater tilapia (Dharmamba *et al.*, 1973). Gill Na^+/K^+-ATPase activity in seawater fishes is also reduced by prolactin treatment (Gallis *et al.*, 1979). Studies on the opercular membranes of killifish and tilapia show that prolactin inhibits the active Cl^- efflux typical for seawater fishes (Mayer-Gostan and Zadunaisky, 1978; Foskett *et al.*, 1982b).

As emphasized by Bern *et al.* (1981), effects of prolactin in fishes develop slowly. This may imply that its effects are exerted on growth and differentiation of branchial cells, at least in freshwater fishes. For seawater tilapia, Foskett *et al.* (1982b) concluded that prolactin decreased the number of opercular C.C. cells.

There is some recent evidence for the presence of prolactin receptors in teleost gills. Edery *et al.* (1984) succeeded in demonstrating specific binding (although with a low capacity) of homologous prolactin to gills of tilapia.

It should be kept in mind that most of the experimental data presented here were obtained with mammalian prolactins. However, injections of fish prolactins (Clarke, 1973; Specker *et al.*, 1985) and implantation of teleost prolactin cell grafts (Wendelaar Bonga and Meis, 1982) have shown that fish prolactin often has similar effects to those of mammalian prolactin on teleost hydromineral regulation.

C. Cortisol

Cortisol is the major corticosteroid secreted by the teleost interrenal gland in freshwater as well as seawater fishes (see Balm, 1986). Only minute amounts of other steroids have been demonstrated, although Weisbart and McGowan (1984) recently showed that cortisone may be the principal interrenal steroid hormone in Atlantic salmon. Cortisol has glucocorticoid as well as mineralocorticoid effects in fish. The gills are an important target organ for cortisol, which stimulates active ion transport (Na^+ and Cl^- in particular) in freshwater and seawater fishes. Recently, DiBattista *et al.* (1984) found evidence for glucocorticoid receptors in the eel gill cytosol.

1. Freshwater Fishes

Chester-Jones *et al.* (1964) realized the first, although partial, interrenalectomy in freshwater eels and observed a reduction of plasma Na^+ levels and a decrease in branchial Na^+ uptake. Low doses of cortisol can restore Na^+ balance in interrenalectomized eels (Chester-Jones *et al.*, 1972). Stimulation

of the Na^+,K^+-ATPase activity following cortisol administration has been reported for several species, including eels, mullet, and tilapia (Epstein *et al.*, 1971; Kamiya, 1972; Forrest *et al.*, 1973; Gallis *et al.*, 1979; Balm, 1986). Recently, Balm (1986) has shown that an Na^+,H^+-ATPase-like enzyme activity, present in the gills of tilapia, is also stimulated by cortisol administration. Since Na^+,K^+-ATPase activity (Claiborne *et al.*, 1982; Evans *et al.*, 1982) and the presumptive Na^+,H^+-ATPase may be regulating Na^+ uptake and H^+ and NH_4^+ extrusion, these observations support the conclusion that in freshwater fishes cortisol has important effects on active Na^+ uptake, acid–base regulation, and nitrogen excretion via the gills. In contrast to prolactin, cortisol does not reduce the passive Na^+ flux across the gills (Dharmamba and Maetz, 1972; Forrest *et al.*, 1973). A stimulation of branchial water influx has been reported for the goldfish (Ogawa, 1975), although at pharmacological doses. However, in the mullet no effect of cortisol on branchial permeability to water could be demonstrated (Gallis *et al.*, 1979).

2. *Seawater Fishes*

In seawater-adapted eels, the plasma Na^+ concentration is increased and branchial Na^+ extrusion decreased following complete interrenalectomy. Cortisol injections can restore plasma Na^+ levels and Na^+ efflux (Mayer *et al.*, 1967). Cortisol injections in seawater-adapted tilapia stimulate Cl^- secretion across the opercular membrane (Foskett *et al.*, 1983). Injections of cortisol increase the Na^+ turnover rate (Mayer and Maetz, 1967) and also increase the Na^+,K^+-ATPase activity and the number of branchial C.C. (Kamiya, 1972; Doyle and Epstein, 1972; Thompson and Sargent, 1977).

Reports on cortisol receptors in the gills are limited. Recently DiBattista *et al.* (1984) found evidence for glucocorticoid receptors in the eel gill cytosol.

Some comments should be made concerning corticosteroid studies.

1. With respect to interrenal function in fishes, only a few species have been studied, and eels are clearly overrepresented. It should be kept in mind that eels are not representative of freshwater teleosts in general, since ion fluxes across the gills are very low. For example, Cl^- influx in the freshwater eel varies from 0.1 to 0.4 μeq/hr per 100 g (Bornancin *et al.*, 1977), while it amounts to 47 in goldfish (De Renzis and Maetz, 1973) and about 20 in trout (Kerstetter and Kirschner, 1972). This perhaps may imply that the role of cortisol is of less importance for freshwater eels than for other freshwater fishes.

2. Cortisol has traditionally been associated with hydromineral control in seawater, but, as mentioned above, the hormone is also important for life in fresh water. Nevertheless, during migration of most euryhaline fishes from fresh water to seawater, cortisol secretion increases, but release of hormone was also observed on the reverse transfer or when fishes were moved from

fresh water to deionized water (Assem and Hanke, 1981). It is also clear that production rates as well as clearance rates are higher in seawater (Leloup-Hatey, 1974; Henderson *et al.*, 1974) and so is the Na^+/K^+-ATPase activity in several species (Kamiya, 1972; Maetz and Bornancin, 1975). However, clearance rates are not related to external salinity in the flounder, and in the mullet the Na^+,K^+-ATPase activity was found to be higher in fresh water than in seawater (Carrick and Balment, 1984; Gallis *et al.*, 1979).

3. Cortisol has been considered to act antagonistically to prolactin. One antagonistic action is undisputed: in seawater fishes cortisol stimulates and prolactin inhibits Na^+,K^+-ATPase activity and Na^+ and Cl^- effluxes. However, in freshwater fishes cortisol and prolactin certainly do not act in an antagonistic manner but rather in a synergistic way at the branchial level, with cortisol controlling active ion exchange and prolactin primarily controlling passive ion fluxes.

4. The existence of a hypothalamo–hypophyseal–interrenal axis is undisputed in fishes. Although ACTH is an important secretagogue for the interrenal, it is certainly not unique. In addition to this, several reports show that ACTH may have effects similar to those of cortisol in hydromineral regulation. Chan *et al.* (1968) observed that both cortisol and ACTH partially restore the low plasma Na^+ levels in hypophysectomized freshwater eels. In seawater animals both hormones are efficient in increasing Na^+ efflux in intact or hypophysectomized animals (Mayer and Maetz, 1967).

D. Calcitonin

The function of calcitonin in fishes is controversial. Injections of mammalian or teleostean calcitonin preparations have been shown to reduce plasma calcium levels in some species, but many attempts to influence plasma Ca^{2+} have been unsuccessful (see Wendelaar Bonga, 1981; Wales and Berrett, 1983). Recently, the plasma calcitonin and the calcitonin content of ultimobranchial bodies were shown to increase for a few days during transfer of trout from fresh water to seawater (Fouchereau-Peron *et al.*, 1986), but in general no clear correlation between plasma Ca^{2+} and calcitonin levels has been observed (Shiraki *et al.*, 1982; Björnsson and Deftos, 1985). This may be caused by the fact that hormonal control of plasma Ca^{2+} is complicated and involves several hormones. Nevertheless, observations on isolated eel gills indicate that calcitonin may be an important Ca^{2+}-regulating hormone in fishes. It stimulates branchial efflux of Ca^{2+} (Milet *et al.*, 1979; Milhaud *et al.*, 1980) and decreases the branchial Ca^{2+} influx (Milet *et al.*, 1979).

Specific receptors for calcitonin have been found in trout gill cells (Fouchereau Peron *et al.*, 1981), and a recent study suggests the presence of a single class of binding sites in trout gill membranes (Arlot-Bonnemains *et al.*, 1983). Although the available evidence is still sparse, the gills seem to be one of the principal target organs for calcitonin in fishes.

E. Corpuscles of Stannius

The corpuscles of Stannius (CS) are small glands connected with the kidney and are typical for holostean and teleostean fishes. It is now clear that they are not homologous to the interrenal bodies. Removal of the glands invariably results in a marked increase of plasma Ca^{2+}. Changes in other ions, such as a decrease in phosphate and magnesium and an increase in K^+, have sometimes been reported. The hypercalcemia can be redressed by reimplantation of the glands or injections of CS extracts (see review by Fenwick, 1982a).

Fontaine *et al.* (1972), Milet *et al.* (1975), and Fenwick and So (1974) have provided evidence that hypercalcemia following removal of the CS in eels is due to alterations in Ca^{2+} exchanges between the fish and the ambient water, which points to the gills as a target organ for the CS. After removal of the glands in eels, isotopic measurements demonstrated an increase in Ca^{2+} influx (So and Fenwick, 1977; Milet *et al.*, 1978) and a decrease in Ca^{2+} efflux (Milet *et al.*, 1978). The changes in Ca^{2+} fluxes could be redressed by injections of CS extracts. Stanniectomy of eels maintained in fresh water (Ca^{2+} about 1 m*M*) resulted in proliferation and hypertrophy of the C.C. and in increased binding of Ca^{2+} to the branchial mucosa (Chartier *et al.*, 1977). The first name proposed for the hypocalcemic factor of the CS was hypocalcin (Pang *et al.*, 1974), tentatively identified as a heat-stable protein with a molecular mass higher than 13,000 daltons. These results have been confirmed by Fenwick (1982b), who found that the active portion of the eel CS is a protein with a molecular mass higher than 10,000 and by Wendelaar Bonga *et al.* (1985) who isolated a glycoprotein from eel and trout with a molecular mass of about 28,000. A second factor, called teleocalcin, a small glycopeptide with a molecular mass of about 3000, has also been isolated (Ma and Copp, 1978). This molecule inhibits the low-affinity phosphatase activity from the plasma membranes of teleost gills, as well as branchial Ca^{2+} uptake in eels (Ma and Copp, 1982). The activity of this enzyme was found to be stimulated after stanniectomy (Fenwick, 1976). These results suggested that this enzyme was connected with Ca^{2+} uptake across the gill. A third name, parathyrin, was given to a hormone contained in the CS that bears immunological similarities to a peptide of terrestrial vertebrates, PTH (Milet *et al.*, 1980). More studies are needed to clarify whether CS produce one or more hypocalcemic factors and how these hormones may act on the gill epithelial cells.

F. Neurohypophysial Hormones

The natural neurohypophysial peptides of teleosts are arginine vasotocin (AVT) and isotocin (IT). Whereas the kidneys seem the principal targets for these hormones, they also have effects on the gills, in particular on hemodynamics. Their action on blood-flow distribution in the gills is reversed from

that of adrenalin: they enhance the blood circulation in the central venous system and the marginal vessels of the secondary lamellae at the expense of the surface of the secondary lamellae (Rankin and Maetz, 1971). In the goldfish, Maetz *et al.* (1964) have observed that Na^+ influx is increased by isotocin, but the amounts of hormone injected were large and, as suggested by Sawyer and Pang (1980), may not have been physiological. AVT has also been shown to reduce the diffusional water flux across the gills, but these effects could not be separated from its hemodynamic effects (Lahlou and Giordan, 1970). Motais and Maetz (1964) showed that injection of AVT accelerates readjustment of Na^+ efflux in flounder when transferred from fresh water to seawater. Recently, Guibbolini *et al.* (1985) observed a decrease of the adenylate cyclase activity of trout and mullet gill cells of 10–50% by AVT and IT in concentrations ranging from 10^{-9} to 10^{-12} *M*, which could modulate the ion transporting capacity of the gills.

G. Urotensins

The urotensins are peptides produced in neuroendocrine cells located in the caudal spinal cord, with processes forming a neurohemal organ, the urophysis. Urotensin I and II are the main putative hormones. The amino acid sequence of urotensin I is closely related to that of the amphibian skin peptide sauvagine and of mammalian CRF. Urotensin II is a dodecapeptide partially homologous to somatostatin-14. Earlier data have indicated that the urophysis is implicated in osmoregulation. Effects have been reported on plasma electrolytes, blood pressure, urine flow, and renal electrolyte excretion (see review by Bern, 1985). The gland has also been shown to respond to the ionic composition of the external medium (Sachs and Chevalier, 1984). Data on the effects of urotensins on gills are scarce. Maetz *et al.* (1964) showed that urophyseal extracts increased branchial Na^+ influx in the goldfish by a substance tentatively called urotensin III, but this observation has not been confirmed. Marshall and Bern (1981) demonstrated that urotensin II inhibits the short-circuit current and increases the resistance of the isolated seawater *Gillichthys* skin preparation, as a result of a reduction of Cl^- excretion by the C.C. Similar results, although obtained at higher doses, have been reported for the opercular membrane of tilapia (Loretz *et al.*, 1981). In the *Gillichthys* skin, inhibition by epinephrine or urotensin II could be reversed by urotensin I (Marshall and Bern, 1981). The urotensins may affect the gills indirectly, such as by modification of the release ACTH (urotensin I) or of prolactin (urotensin II; see review by Bern, 1985).

H. Thyroid Hormones

Although there are indications, especially for migratory species, that thyroid hormones are connected with osmoregulation, the relationship is far from conclusive (see review by Leatherland, 1982). Smolting of salmon in-

volves an increase of C.C. and branchial Na^+,K^+-ATPase activity, but these changes occur weeks after a plasma thyroxin surge (Loretz *et al.*, 1982) and there is no evidence for a direct involvement of the thyroid gland. Reports showing that gill Na^+,K^+-ATPase activity in *Oncorhynchus kisutch* increased after administration of low doses of thyroxine have not been confirmed (Leatherland, 1982). Shivakumar and Jayaraman (1984) have suggested that thyroxine regulates branchial mitochondrial function during adaptation of fishes to hyperosmotic water, possibly in part by promoting mitochondrial protein synthesis.

I. Other Hormones

For several other hormones effects have been reported on the gills. Most of these hormones are rapid-acting and have been studied only in seawater fishes. Somatostatin was shown to inhibit the short-circuit current and to reduce the tissue conductance of opercular membranes (Foskett *et al.*, 1981). Glucagon and vasoactive intestinal polypeptide (VIP) have been reported to stimulate Cl^- secretion of opercular membranes (Foskett *et al.*, 1982a) and isolated gill arches (Davis and Shuttleworth, 1985). Glucagon and VIP both stimulate the adenylate cyclase activity of gill membrane–enriched fractions at concentrations of 10^{-12} *M* (M. Guibbolini and B. Lahlou, unpublished data) and can thus be considered to act via cAMP as second messenger.

V. CONCLUDING REMARKS AND POSSIBLE BIOMEDICAL IMPLICATIONS

In the preceding sections, the literature on hormonal actions at the gill level has been reviewed for freshwater and seawater fishes. It appears that our present knowledge of the endocrine involvement in gill function is based on a relatively small sample of fish species, which includes a disproportionally high number of euryhaline species. Although solid data are missing, there are indications that the involvement of hormones in the regulation of gill function is more pronounced in euryhaline than in stenohaline fishes, and therefore any generalization should be considered with caution.

Whereas the ionic composition of full-strength seawater used by different authors is probably rather similar, large variations undoubtedly exist in the ionic composition and pH of the fresh water used. Differences in water composition, in particular in the concentrations of monovalent ions, Ca^{2+}, and water pH, have multiple effects, direct as well as indirect, on gill transport and, consequently, on parameters such as TEP, ion-dependent ATPase activity, density of C.C., and the activity of the hormonal tissue implicated in the control of branchial structure and function. Possibly, several discrep-

ancies in the data presented above have been caused by such differences in experimental conditions. This may apply for example, to the data on the differences reported for the location and density of C.C. and of branchial Na^+/K^+-ATPase activity for euryhaline fishes from seawater and fresh water. The differences will certainly be influenced by the ion composition of the fresh water used in such experiments.

Information collected during the last decade has resulted in a new concept of the function of the C.C. in seawater fishes that finds wide support. It is based on the assumption that Na^+,K^+-ATPase is the driving force for Cl^- extrusion. Recent models indicate that Cl^- transport occurs by a transcellular route followed by the passive movement of Na^+ via paracellular channels (Karnaky, 1986).

The function of the C.C. in freshwater fishes is still controversial, and it has even been suggested that the C.C. in freshwater fishes are largely nonfunctional or degenerative. As reported above, reduction of opercular C.C. numbers by cellular involution following transfer of fishes from seawater to fresh water has indeed been observed, for example, for tilapia. However, the numbers of C.C. in fully adapted freshwater fishes are influenced by many water parameters. In tilapia and trout we observed C.C. densities in fresh water with low Ca^{2+} concentrations or low pH that were much higher than observed in fishes from full-strength seawater. The same observation was made for the number of cortisol-producing interrenal cells (Wendelaar Bonga and Balm, 1987). With respect to C.C. function and its endocrine control, however, these observations are all circumstantial and the need for direct evidence is obvious.

Fish gills are organs that are complicated and highly specialized for an aquatic way of life, and therefore direct biomedical advances can not be expected from studies on gill function. There is no doubt, however, that fish gills and opercular epithelia are exceptionally good models for studying the endocrine control of water and electrolyte transport across cellular membranes. Whereas in studies on such membranes in mammals it is difficult to alter the composition of the fluid compartments, with fish gills the compartments can be changed easily, and the gills can tolerate a wide range of salinities. The opercular membranes can be mounted in chambers, and this is a great advantage when hormones and other substances are applied and their effects assessed. Because of these experimental possibilities these fish tissues are of interest for all investigators studying water and electrolyte transport, in particular those that are interested in Cl^- transporting membranes in mammals, such as the ascending limb of the loop of Henle or the cornea. In addition, all vertebrates have, for a shorter or longer time, to experience the aquatic life and some epithelia in the body have in fact to face situations which are quite similar to those of the gills with an ionic mucosal fluid concentration different from that of plasma.

In conclusion, the fish gill, which can fulfill some functions of the gut and

the skin, most of that of the kidney, and all those of the lungs, is an extraordinary biological material for physiological, pharmacological, and endocrinological studies.

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